Rev. Écol. Biol. Sol, 1991, **28** (3) : 287-302.

Microstructural and ultrastructural changes of the oak leaf litter consumed by millipede *Glomeris hexasticha* (Diplopoda)

BY

K. TAJOVSKÝ (*), G. VILLEMIN (**) and F. TOUTAIN (**)

(*) *Institute of Soil Biology, Czechoslovak Academy of Sciences, Na sádkach* 7, 370 05 *České Budějovice, Czechoslovakia*
(**) *Centre de Pédologie Biologique, CNRS, BP* 5, *Vandœuvres-lès-Nancy Cedex, France*

Synopsis: The microstructural and ultrastructural changes of oak leaf litter during its passage through the digestive tract of *Glomeris hexasticha* and subsequent decomposition of the faecal pellets of this millipede in field conditions were studied. Millipedes disintegrated leaf litter and significantly influenced microbial communities of the ingested food. The decomposing faecal pellets were joined by fungi and roots. Degradation of plant fragments as well as microbial activities were observed by the scanning and transmission electron microscopy.

Keywords: Diplopoda, millipede, *Glomeris hexasticha*, oak leaf litter, gut content, faecal pellets, microstructure, ultrastructure, microorganisms, brown pigments.

INTRODUCTION

Millipedes (Diplopoda) are able to consume large amounts of dead plant material, which they release imperfectly digested back into the soil. Fragmentation and comminution of litter, mixture of organic debris with mineral particles and transport into deeper layers of litter and soil in the shape of well distinguished faecal pellets are a contribution to the decomposing processes (*cf.* CROSSLEY, 1977). Faecal pellets including microorganisms, products of metabolism and partially digested plant residues are the foci of high biological activity in soil environment.

Reçu le 6/11/1990.
Accepté le 24/7/1991.

Many papers paid attention to the feeding biology and function of millipedes in humification (GERE, 1956; BOCOCK, 1963; VAN DER DRIFT, 1975, etc.), as well as to microbial and chemical aspects of ingested food (HANLON, 1981 *a*), gut content (TAYLOR, 1982; TAYLOR & CRAWFORD, 1982) and excrements (MCBRAYER, 1973; HANLON, 1981 *b*; INESON & ANDERSON, 1985). The studies of faecal pellets decomposition and comparisons with the changes in the consumed litter contributed to the understanding of the importance of millipedes in humification and soil forming processes, too (NICHOLSON *et al.*, 1966; WEBB, 1977; HANLON, 1981 *a*, *b*; ANDERSON *et al.*, 1983 *a*).

Only some authors studied the microstructure and ultrastructure of the gut content and excrements of soil invertebrates. Transmission and scanning electron microscopy were used for the study of food diet of collembolans (KILBERTUS & VANNIER, 1978, 1979, 1981; SAUR & PONGE, 1988). DELEPORTE (1988) described the ultrastructural changes of plant residues in the gut of larvae *Bradysia confinis* (Diptera, Sciaridae). BIGNELL (1989) demonstrated by the scanning electron microscopy the presence and changes of microorganisms in food, gut contents and excrements of *Glomeris marginata*. The ultrastructure of decomposing faecal pellets is not sufficiently known. On the contrary, much information exists about the ultrastructure of decaying plant residues as for example leaf litter, roots, wood, etc. (REISINGER *et al.*, 1971; TOUTAIN, 1981; FRANÇOIS *et al.*, 1986; BARAER & TOUTAIN, 1987).

In this work we are dealing with the micro- and ultrastructure of leaf litter, gut content and decomposing faecal pellets of the millipede *Glomeris hexasticha* Brandt, 1833.

I. – MATERIAL AND METHODS

The material of millipedes and leaf litter was collected in the experimental plot near Netolice in South Bohemia (Project VII-1-2 "Biological structure and function of the soil part of the ecosystems" of the Institute of Soil Biology, Czechoslovak Academy of Sciences). The locality is covered with mixed deciduous forest with dominanting 70-years-old oak trees (*Quercus robur* L.). *Glomeris hexasticha* is eudominant species in this forest and its main food resource is decaying oak leaf litter (TAJOVSKÝ, 1989).

Millipedes and wet 6 or 7-month-old brown oak leaves were placed in laboratory into glass vessels. Animals consumed leaves and released excrements. Structural changes were investigated in the initial leaf litter consumed, in the gut contents, freshly produced pellets and on the faecal pellets of different stages of decomposition. Through the preparation of five animals we obtained the guts filled with food. The gut of millpedes of the genus *Glomeris* do not form straight tube, but it is situated in the animal body in the shape of turned-up N type. The first direct part is formed by short foregut (oesophagus) and extended midgut. Behind the pylorus, the hindgut returns by ileum and a part of colon to the anterior body end. The colon comes back and leads into rectum. Short oesophagus was at dissected individuals without food. The gut content was studied in midgut, ileum and colon. For the fresh faecal pellets preparation, several animals were displaced into empty vessel. Released pellets were immediately processed. Field decomposition experiment, from which the further material under study was taken, was aimed at some physical, chemical and biological characteristics of the pellets of *Glomeris hexasticha* during its 1 year exposure in small microcosms in soil of the experimental plot near Netolice (TAJOVSKÝ *et al.*, in press). Beside the initial stage (time 0), the excrements from the 7th, 20th, 48th, 124th, 209th, and 370th days of decomposition were investigated. They were always after sampling subsequently freeze dried and stored under laboratory conditions.

Three methods were used for the study of microstructural and ultrastructural changes of leaf litter. The whole spectrum of the material under study was processed by transmission electron microscopy (TEM). Fresh faecal pellets were prepared according to VILLEMIN and TOUTAIN (1987). The other material (leaf litter, guts and decomposing pellets) were prefixed by 2.5% phosphate buffered glutaraldehyde for 16 hours, fixed with 2% phosphate buffered OsO_4 for 1 hour, and dehydrated through a graded series of acetone. The material was embedded in Polarbed 812 resin. The ultrathin sections were colored by uranylacetate and lead citrate and examined using a Carl Zeiss EM 9-S-2 electron microscope (60 kV, University of Nancy). The decomposing faecal pellets were studied by scanning electron microscopy (SEM), too. Small specimens of freeze dried pellets were coated with gold, and examined using a Tesla BS 300 electron microscope (19 kV, Electron Miscroscopy Laboratory, České Budějovice). Soil thin section method (JONGERIUS, 1964) was used for evaluation of microstructure of the decomposing pellets, too. Excrements were impregnated under vakuum by CHS 104 epoxyde resin of Czechoslovak production. Thin sections were examined under polarized and non polarized light.

II. – RESULTS

A) Leaf litter

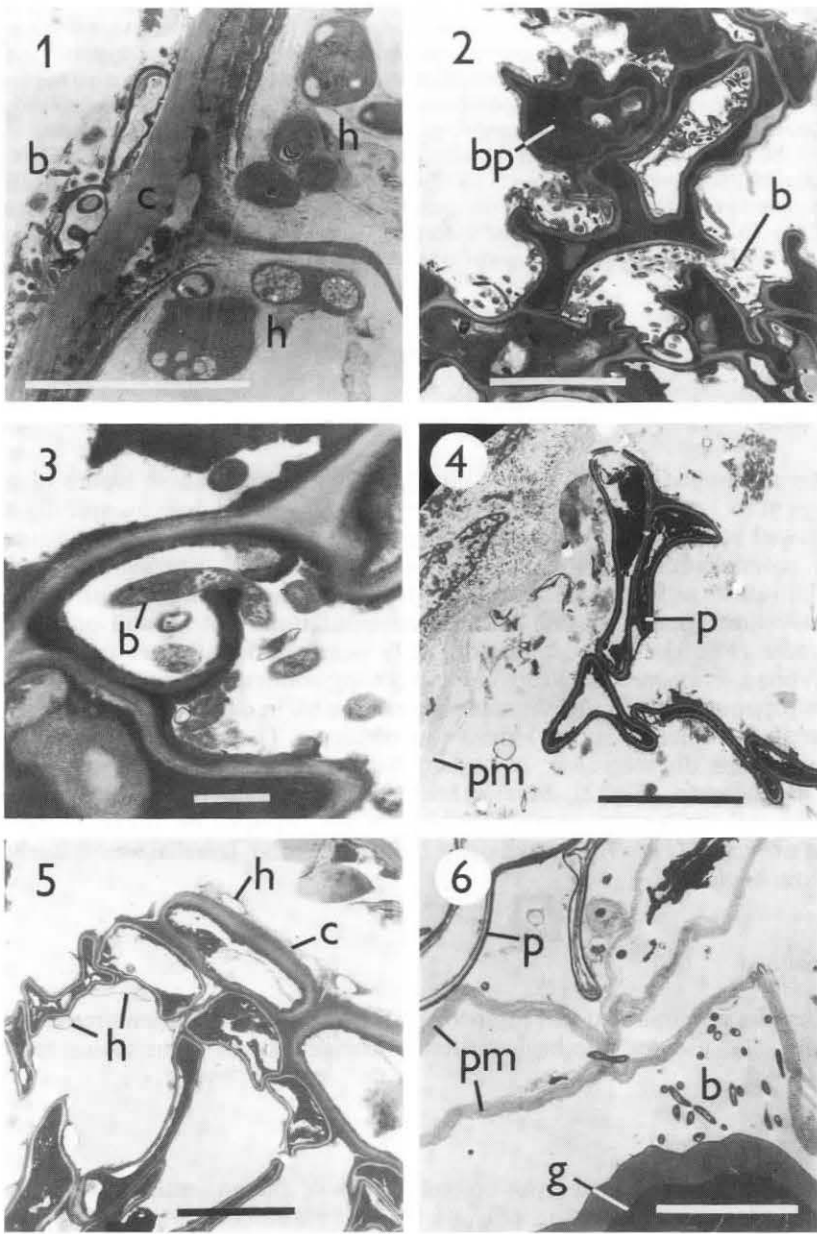
Litter consumed by millipedes contained fragments or whole blades of brown oak leaves. 6 to 7-month-old overwintering leaf litter seemed macroscopically entire, but it showed some visible degradation changes. On the cross section, the epidermis, palisade parenchyma, spongy parenchyma and vascular systems were well-preserved. Ultrathin sections showed a level of the passing decomposition. Superficial fungal mycelium as well as rich bacteria accumulations were found on the upper leaf surfaces (*Fig.* 1). Upper epidermal cells were abundantly grown through by fungal hyphae. Transmission electron miscroscopy confirmed the presence of brown pigments (opaque areas in the electron field) above all in dark brown leaves (*Fig.* 2). They were less numerous in the light-coloured leaves. These pigments were in some cells nearly quite disintegrated. Abundant bacteria were observed in palisade and spondy parenchyma (*Fig.* 2). Several bacteria were found in the brown pigment drops. The cell walls were conserved, but the perforations by bacteria and fungi could be observed (*Fig.* 3). Sporadically the intercellular lamella was disturbed by pectinolytic bacteria.

B) Gut content

Millipedes comminuted leaf litter to small fragments (maximum area 0.2 mm^2). In the majority of cases, the basic structure arangement of plant tissues remained well-preserved.

Midgut

The food was separated from epithelial cells of the gut wall by several strata forming peritrophic membrane (*Fig.* 4). Many plant cells were broken, pectic intercellular lamella and parts of cell walls were locally lysed. Cuticle of the epidermal cells was disturbed (*Fig.* 5). In comparison with the initial litter stage, the perceptible decrease of bacteria and micromycetes was noted here. Only empty hyphae of dead micromycetes and superior fungi and lysed bacterial cells were observed.


1
b
c
h
h
2
bp
b
3
b
4
p
pm
5
h
c
h
6
p
pm
b
g

Hindgut

In the first part of hindgut (ileum), the gut content was still enclosed in a peritrophic membrane (*Fig.* 6), but now no more entire. Aggregations of gut bacteria in the proximity of the gut intima were shown in TEM sections (*Fig.* 6). Dead bacterial cells and rests of hyphae, partially degraded brown pigments and degraded pectic lamella were characteristic for the plant material here examined (*Fig.* 7).

In colon, the plant fragments did not expressively differ from that in the previous part of hindgut. Only dead fungal hyphae were present here. Bacterial cells, probably symbiotic, penetrated into food (*Fig.* 8).

In the last gut part (rectum), the gut content is depressed and the faecal pellets are formed here. The most expressive mechanical influence on the food material is probably in this part of digestive tract. The shape of rectum predetermines the future typical form of released pellets (*Fig.* 9). Most expressive mechanical deformations of the plant cell walls as well as plant tissues were really well visible as from the fresh faecal pellets (*Fig.* 10). Peritrophic membrane was not observed here.

C) Faecal pellets

The faecal pellet of *Glomeris hexasticha* has a shape of a smooth truncated cone with a small tip on wider base (*Fig.* 9). The pellets used in our experiment had size range of 1.6-2.0 × 1.2-1.4 mm and dry weight 0.40 mg in average. Particular building components were distinguishable on their surfaces, but the peritrophic membrane and/or at least its rests were not observed.

Ultrathin sections of freshly released pellets confirmed still high density and activity of bacteria. As regards mycromycetes, it was possibe to find only empty dead hyphae. Fresh excrements resembled the material from the posterior part of hindgut. Mechanical deformations of cell walls and of spongy parenchyma were well visible. Dead plant cells contained plenty of brown pigments (*Fig.* 10).

In substance, the inner structure of the material from the time 0 of decomposition was well demonstrated on the thin sections. Particular leaf fragments orientated parallely with pellet surface determined its rounded shapes. The fragments were mostly staggered in the whole pellet and had well-preserved tissue structures (*Fig.* 11). Brown pigments were visible in light microscopy in cells as granules and

---

FIG. 1. – Leaf litter: Upper epidermal cells and cuticle with bacteria and fungal hyphae. Bar = 10 μm.

FIG. 2. – Leaf litter: Opaque areas in the electron field (probably brown pigments) and bacteria in the spongy parenchyma. Bar = 10 μm.

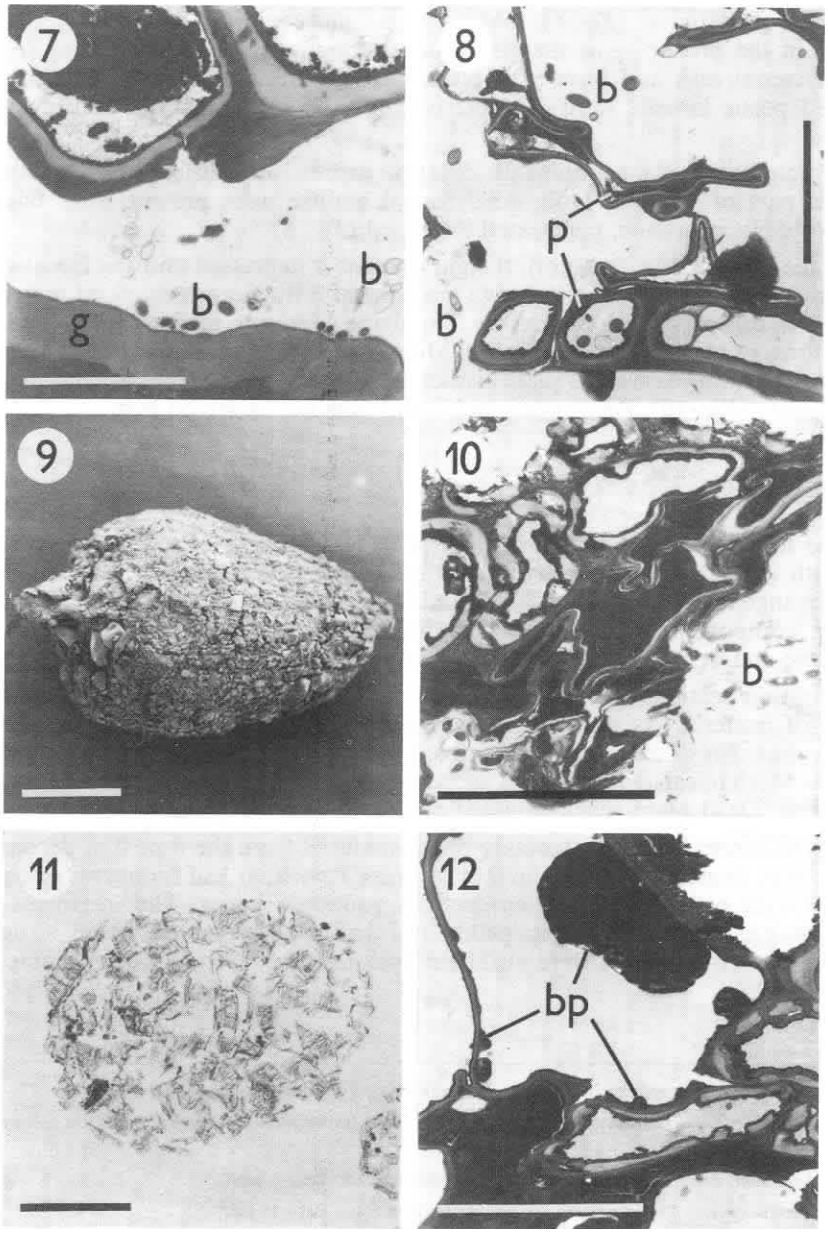
FIG. 3. – Leaf litter: Perforation of cell wall by cellulolytic bacterium. Bar = 1 μm.

FIG. 4. – Midgut content: Plant fragment and peritrophic membrane. Bar = 10 μm.

FIG. 5. – Midgut content: Plant fragment with empty fungal hyphae. Bar = 10 μm.

FIG. 6. – Hindgut content: Ileum. Symbiotic bacteria in the ectoperitrophic space, peritrophic membrane and plant fragments. Bar = 10 μm.

Abbreviations: *b* = bacteria, *bp* = brown pigments, *c* = cuticle of epidermal cells, *g* = gut wall, *h* = fungal hyphae, *p* = plant fragments, *pm* = peritrophic membrane.


7
b
b
g
8
b
p
b
9
10
b
11
12
bp

thin layers along the inner cell walls. Only lignify cell walls of the vascular systems and fine mineral grains glistened in the polarized light. TEM sections showed the presence of dead melanized fungal hyphae and local bacterial accumulation. Figure 12 shows the location of brown pigments along the walls and in the shape of granules in the TEM sections.

After 7 days of decomposition, the pellets laid still separately. The mycelial mat covered their surfaces, but did not join them together. On the thin sections the pellets were compact and entire. The inner microstructure did not alter either. Higher amount of fungal hyphae in the pellets was connected with the development of micromycetes. Brown pigments were more frequently present in the granular form (*Fig.* 13). Deepening cellulolysis of the cell walls in some parts of palisade and spongy parenchyma was perceptible. Bacteria, dead and alive hyphae were present.

On the 20th day of decomposition, some pellets were partially deformed. Thin sections showed, that the cell deformations of the palisade and spongy parenchyma were deeper then. The cuticle layer of the epidermis was more destroyed. The fungal mycelium was well visible. The ultrastructure presented no more expressive changes in the comparison with previous specimens. The brown pigments were furthermore abundant.

After 48 days, the pellets were partially bound by fungal hyphae and also by fine plant roots. In many pellets, an expressive decrease of the fine amorphous material among the plant fragments was observed (*Fig.* 14). Although on the thin sections the pellets rested more or less compact and only little deformed, the cross TEM sections of leaf fragments demonstrated a desintegration of lower epidermis cells and passim spongy parenchyma cells, too. Melanized hyphae were well visible in light microscopy, too.

Most excrements were bound with fungal mycelium and roots into bunch-like formations and they were expressively deformed after 124 days of decomposition. The material maintained this character during the whole experiment duration (*Fig.* 15). On the thin sections, coarse plant fragments stood out from the pellet surfaces. Leaf fragments were henceforth well distinguishable with a differently proved tissues. The least structural changes were mostly in the upper epidermis with cuticle and palisade parenchyma. The cell walls of vascular systems glistened less than in previous samplings. Pectinolytic and cellulolytic changes of the cell walls were visible on the TEM sections (*Fig.* 16, 17). During the biodegradation of the melanized mycelial filaments (*Fig.* 17), the fine granules formed on their surfaces (*Fig.* 18). The zones with brown pigment degradations were not frequent. Bacteria were found in these pigments sporadicaly (*Fig.* 19).

FIG. 7. – Hingut content: Dead and alive bacteria and partially degraded plant material. Bar = 5 μm.

FIG. 8. – Hingut content: Colon. Plant fragments with symbiotic bacteria. Bar = 10 μm.

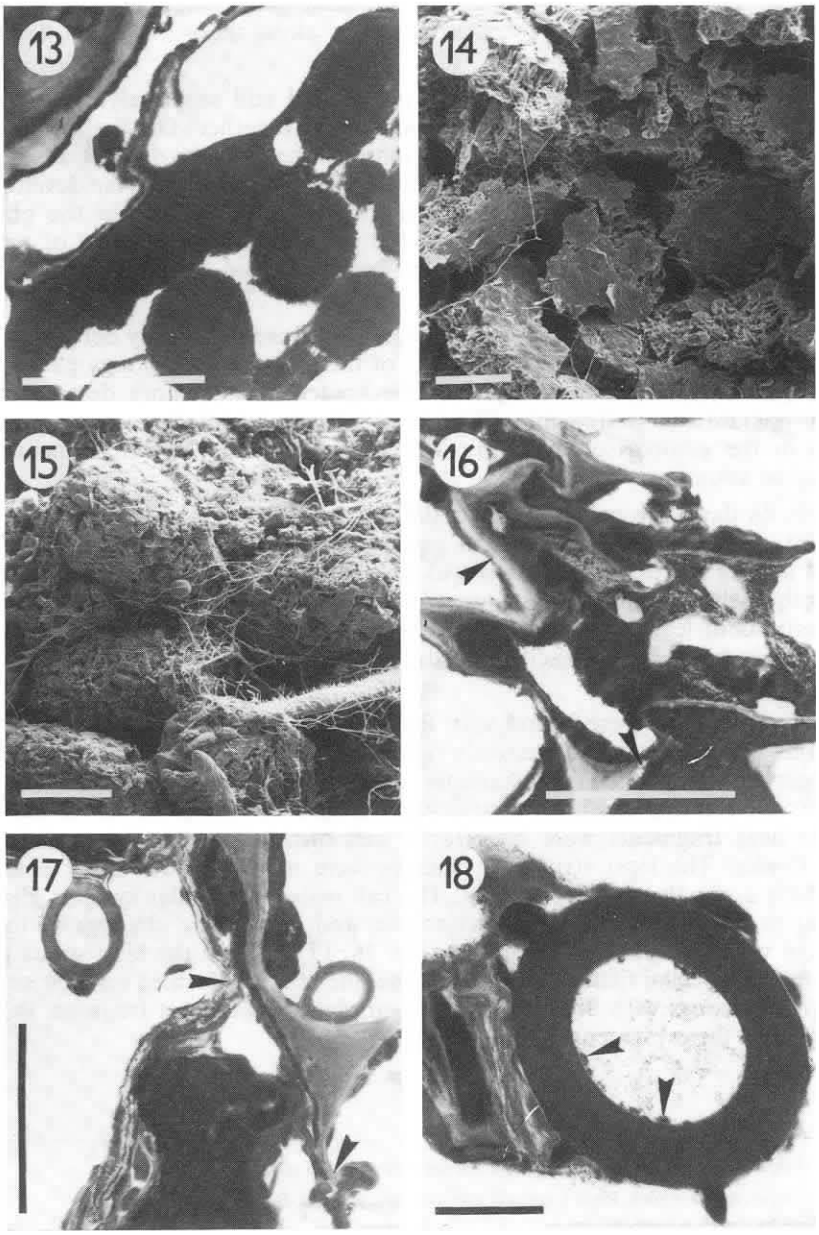
FIG. 9. – Faecal pellets: Faecal pellet of *Glomeris hexasticha*. Bar = 500 μm.

FIG. 10. – Faecal pellets: Fresh faecal pellets. Deformed plant cells with bacteria. Bar = 10 μm.

FIG. 11. – Faecal pellets: Thin cross section of faecal pellet (0 day of decomposition). Bar = 500 μm.

FIG. 12. – Faecal pellets: Brown pigments as granules and thin layer along the cell walls (0 day). Bar = 10 μm.

Abbreviations: *b* = bacteria, *bp* = brown pigments, *g* = gut wall, *p* = plant fragments.


13
14
15
16
17
18

In the time of 209 days in spite of expressive deformations, most of pellets did not quite disintegrate. Coarse and undecomposed plant residues rested as a skeleton of initial pellets (*Fig.* 20). On the thin sections they were deformed, fragmented and irregular. Single components were often accumulated and depressed. The cell structures of leaf fragments were little perceptible. Fragments of vascular systems and cuticle layers of upper epidermis remained more compact and distinct than other tissues. In the polarized light, some more compact parts joining the fragments of pellets suggested casts or excrements of other small soil animals, probably enchytraeids. The pectinolysis and cellulolysis of intercellular lamella and cell walls as well as biodegradation of fungal filaments and brown pigments deepened (*Figs.* 21, 22).

After 370 days, the deformed and nearly destroyed faecal pellets made up cluster, and the polarized light confirmed again the possibility of the transformation of some faecal material by other soil saprophagous invertebrates. Still visible small leaf fragments had well-preserved structure of upper epidermis, some parts of palisade parenchyma and the vesicular cells (*Fig.* 23). The material was generally lighter coloured than before. On the thin sections it was visible, that the brown pigments were missing along cell walls, as well as in the granular form. This fact was noted on the TEM sections, too. Most materials were less opaque in the electron field. The brown pigments were present only in a small amount in some tissue fragments as small particles (*Fig.* 24).

III. – DISCUSSION

The study of microstructure and ultrastructure confirmed the activity of the millipede *Glomeris hexasticha* consists not only in the fragmentation of leaf litter. Expressive changes were found as regards consumed microflora as well as suggestions of the plant material decomposition. The majority of bacteria and micromycetes of the ingested food was assimilated in the midgut. KILBERTUS and VANNIER (1979, 1981) mentioned that fungal, bacterial or algal cytoplasms were the main nutrient sources for the collembolan species and that Collembola control microbial populations rather than exert a direct influence on ingested plant material. SAUR and PONGE (1988) showed that these animals are able to assimilate directly some substances and utilise them as the sources of food. Rich enzymatic equipment facilitating hydrolysis of some components ingested food was found in the millipede

FIG. 13. – Faecal pellets: Granular form of brown pigments on the 7th day of decomposition. Bar = 5 μm.

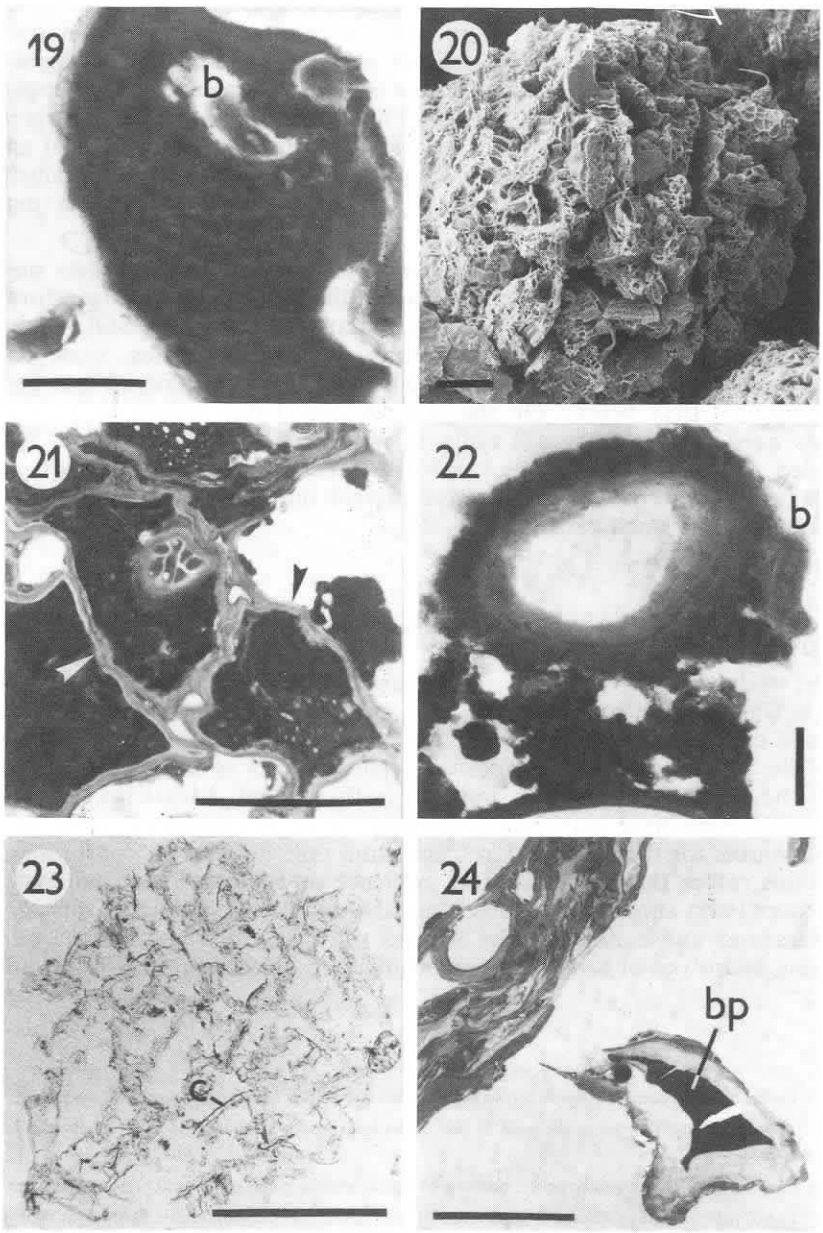
FIG. 14. – Faecal pellets: Expressive decrease of fine amorphous material among the plant fragments (48th day). Bar = 100 μm.

FIG. 15. – Faecal pellets: Decomposing pellets bound with fungal mycelium and plant roots (124th day). Bar = 500 μm.

FIG. 16. – Faecal pellets: Biodegradation of plant material after 124 days of decomposition. Cellulolytic changes of cell walls (arrows). Bar = 5 μm.

FIG. 17. – Faecal pellets: Biodegradation of cell walls (arrows) and dead melanized fungal hyphae (124th day). Bar = 5 μm.

FIG. 18. – Faecal pellets: Biodegradation of melanized mycelial filaments, granulation on the surfaces (124th day). Bar = 1 μm.


19
b
20
21
22
b
23
c
24
bp

guts, too (NIELSEN, 1962; MARCUZZI & TURCHETTO LAFISCA, 1976; BECK & FRIEBE, 1981). Most of the cellulose and hemicellulose degradation due to rich aerobic microbial populations occurred in the millipede midgut, whereas the hindgut was an important site for pectin degradation for the millipede *Orthoporus ornatus* (TAYLOR, 1982). The gut microflora is consequently considered as a main source of enzymatic activity. In the midgut, where epithelial cells are separated from gut contents only by a peritrophic membrane, there is an obvious potential for bacteria to contribute importantly to digestion (CRAWFORD *et al.*, 1983). Nevertheless, the enzymatic activity was found directly in the gut wall, above all midgut (NUNEZ & CRAWFORD, 1976). The presence of active enzymes xylanase, alfa-amylase and cellulase was indicated in the gut wall of *Glomeris hexasticha*, too (URBÁŠEK & TAJOVSKÝ, in press). So in our case both animal and gut microorganisms can take part in degradation of some components. Ulstrastructural studies of gut content showed some changes as gradual decomposition of the cellulose and hemicellulose of the cell walls, decomposition of the pectic intercellular lamella, etc.

BIGNELL (1989) experimentally demonstrated that microorganisms colonizing leaf litter are a valuable nutritional resource to detritivorous animals such as the millipede *Glomeris marginata* and are assimilated with high efficiency. Our results confirmed these facts to a large extent. ANDERSON and BIGNELL (1980) supported the hypothesis that the increased counts of microflora in *Glomeris marginata* faeces result from the proliferation of the indigenous litter bacteria rather than a population of specific gut symbionts. Lately BIGNELL (1989) mentioned that microbial colonizers of gut contents were stripped from leaf litter on ingestion, but also that recolonization of faecal pellets by bacteria occurred during or immediately after transit of the hindgut. TEM showed in our experiment that in the digestive tract of the millipede *Glomeris hexasticha* the alteration of microbial communities takes place owing to bacterial and fungal lysis and subsequent recolonization by gut microbial symbionts.

It is known that faecal pellets of soil saprophagous invertebrates differ from the initial consumed food by some physical and chemical characters and they form a more suitable environment for colonization with bacteria than fungi (MCBRAYER, 1973; HANLON & ANDERSON, 1980; JAMBU *et al.*, 1988). On the soil surface they do not rest a long time without changes and are liable to further decomposing processes (NICHOLSON *et al.*, 1966; WEBB, 1977). The decomposition of studied faecal pellets passed under conditions which made direct influence of soil macrofauna impossible (TAJOVSKÝ *et al.*, in press). Consequently, the biodegradation of these pellets was formed especially by microorganisms, micro- and mesofauna. The plant roots the

FIG. 19. – Faecal pellets: Biodegradation of brown pigments by bacteria. Bar = 1 μm.

FIG. 20. – Faecal pellets: Coarse undecomposed plant residues as a skeleton of initial pellets (209th day). Bar = 100 μm.

FIG. 21. – Faecal pellets: Lysis of cell walls and intercellular lamella (arrows) (209th day). Bar = 5 μm.

FIG. 22. – Faecal pellets: Bacterial degradation of melanized fungal filament and brown pigments (209th day). Bar = 1 μm.

FIG. 23. – Faecal pellets: Thin cross section of faecal pellets in the 370th day of decomposition. Bar = 500 μm.

FIG. 24. – Faecal pellets: Presence of brown pigments only as a small particles in some tissue fragments (370th day). Bar = 10 μm.

Abbreviations: *b* = bacteria, *bp* = brown pigments, *c* = cuticle of epidermal cells.

influence of which it was necessary to consider, too, grew through the microcosms containing pellets. The features of some parts of the pellet material in the two last samplings (209th and 370th days) suggested the cast or excrement structure of some soil animals as for example enchytraeids, which were capable to penetrate into microcosms.

Subsequent degradation of the larger leaf fragments with more or less well-preserved structure of all tissues proceeded mostly from a disturbance of the lower epidermal cell layer ans spongy parenchyma in the direction to palisade parenchyma. After 1 year exposition of pellets in the field, only the structure of upper epidermis or epidermis and palisade parenchyma was often conserved. Cuticle layer was more resisting to the degradation, so that it was well visible on the thin sections in the end of the experiment. Durability of this layer is given by its chemical composition (cutin). Brown pigments are the products containing mainly nitrogen and are relatively resistant to mineralization. Earthworms and white rot fungi are considered the primary metabolizers of these complex compouds. Bacteria, enchytraeids and microarthropods seem to play a minor role in biodegradation of these compouds (TOUTAIN, 1987). Brown pigments were presented along inner cell walls, as granules or quite filled up some cells. The results of the transmission electron microscopy of different decomposition phases confirmed the fact that microorganisms as bacteria, too, participate in the degradation of brown pigments. In the overwintering rests of faecal pellets (samples from the 370th day) only a small amount of brown pigments rested along walls or in the shape of minute fragments in the basic matrix. This decrease was apparently connected in a high degree with chemical changes and subsequent dissolvation by water. The pellets were realy lighter coloured after the winter period. The bacteria were active nearly in all parts of the plant material. They were observed not only in brown pigments, but also in cell walls (cellulolytic bacteria), in pectic intercellular lamella (pectinolytic bacteria) as well as in many other parts of plant rests. They took part in the biodegradation of dead mycelial filaments, etc.

IV. – CONCLUSIONS

The objective of our study were the microstructural and ultrastructural changes of oak leaf litter during its passage through digestive tract of the millipede *Glomeris hexasticha* and subsequent decomposition of the faecal pellets of this millipede in field conditions.

Leaf litter was disintegrated by millipede on minute fragments and very little digested. This fragmentation, indisputedly important for further course of decomposition, appears above all as mechanical transformation of plant remainders. Transmission electron microscopy at the same time showed that millipedes significantly influenced the microbial community of the ingested food. The abundant litter microflora was assimilated so much that in the gut bacterial communities were rebuilt. The primary bacterial cells were mostly lysed and symbiotic gut bacteria presented along the hindgut wall successively penetrated into gut contents. Simultaneously the mycoflora was assimilated and only dead fungal hyphae rested in the ingested material.

Faecal pellets were bound during the decomposition by fungal mycelium and by growing through plant roots. The loss of the fine amorphous material among

the coarse plant fragments was a further characteristic feature of the decomposing pellets. Nevertheless after 1 year's exposure in the soil they did not quite disintegrate. Microstructural and ultrastructural studies showed that degradation of leaf fragments in pellets proceeded mostly from the lower epidermal cells accross spongy parenchyma in the direction to palisade parenchyma. Although cell structure was in the end of the experiment often little visible, the fraction of vascular system as well as upper cuticle layer with epidermal cells were still distinguishable from other decaying material. Microbial activity especially of bacteria was observed in all phases of the decomposition. On the TEM sections they were presented in the cell walls, intercellular lamella, cell lumens as well as dead fungal hyphae. Irregularly the bacterial cells were found in the brown pigments which gave evidence about the ability of these microorganisms to decay those compounds.

The passage of plant material through digestive tracts of soil saprophagous invertebrates such as millipedes means the modification of decomposition processes on the mechanicaly and microbiologicaly changed material. These results must be studied also by another methods (global or analytic methods). The influence of millipede on the microbial communties in the consumed food will be more important than it was considered up to this time. The studies of these interactions between soil invertebrates and microorganisms during decomposition processes will be henceforth important.

Summary

The aim of this study was to show the microstructural and ultrastructural changes of oak leaf litter during its passage through the digestive tract of the millipede *Glomeris hexasticha*, and in its faecal pellets during 1 year decomposition in the soil. Millipedes consume and disintegrate leaf litter and significantly influence microbial communities of the ingested food. The bacterial cells as well as the content of fungal hyphae were assimilated in the gut. Probably symbiotic bacteria penetrated in the hindgut into digested food. During the decomposition the faecal pellets were joined by fungal mycelium and roots into bunch-like formations. The fine amorphous material among the plant fragments decreased and pellets were successively deformed. Microorganisms, especially bacteria, were observed in all phases of the decomposition. On the TEM sections they were active in the cell walls, intercellular lamella, cell lumens as well as in dead fungal hyphae. Irregularly the bacterial cells were found in the brown pigments which give the evidence about the ability of these microorganisms to decay these components, too. Ultrastructural studies of faecal pellets confirmed the probable importance of these units in the soil environment as places with high biological activity.

Résumé

Modifications micro- et ultrastructurales de la litière de feuilles de chêne consommée par le diplopode *Glomeris hexasticha*

Les auteurs se proposent dans cette étude de suivre les transformations micro- et ultrastruturales subies par une litière constituée de feuilles de chêne durant son passage dans le tube digestif fu diplopode *Glomeris hexasticha* et par les boulettes fécales de ces animaux durant une année dans le sol. Ces diplopodes consomment et désintrègrent les feuilles de la litière et influencent la flore microbienne de la nourriture ingérée. Les cellules bactériennes

et les hyphes mycéliens sont assimilés dans l'intestin. Toutefois dans la partie postérieure, on retrouve des bactéries vivantes, probablement symbiotiques, dans la nourriture digérée.

Durant la décomposition dans le sol, les boulettes fécales sont rattachées les unes aux autres par un réseau de filaments mycéliens et de racines fines. Le matériel emorphe et fin qui se trouve entre les fragments végétaux disparaît et les boulettes fécales se déforment peu à peu. Les microorganismes, spécialement les bactéries, sont actifs dans toutes les phases de ces transformations et l'observation en microscopic électronique à transmission permet de souligner leur activité au niveau des parois cellulaires, des lamelles intercellulaires, dans les lumières des cellules aussi bien que dans les hyphes fongiques morts. Çà et là, des cellules bactériennes se rencontrent à l'intérieur de pigments bruns ce qui souligne la possibilité pour ces organismes de biodégrader ces composés résistants.

Les études ultrastructurales des boulettes fécales soulignent l'importance de ces micro-sites spécifiques dans l'environnment du sol en tant que sites de grande activité biologique.

References

Anderson (J. M.) & Bignell (D. E.), 1980. – Bacteria in the food, gut contents and faeces of the litter-feeding millipede *Glomeris marginata* (Villers). *Soil Biol. Biochem.*, **12** : 251-254.

Anderson (J. M.), Ineson (P.) & Huish (S. A.), 1983. – Nitrogen and cation mobilization by soil fauna feeding on leaf litter and soil organic matter from deciduous woodlands. *Soil Biol. Biochem.*, **15** : 463-467.

Baraer (M.) & Toutain (F.), 1987. – Importance des pigments bruns dans les racines superficielles du hêtre (*Fagus silvatica* L.) et biodégradation dans le sol. *Rev. Écol. Biol. Sol.*, **24** : 431-441.

Beck (L.) & Friebe (B.), 1981. – Verwertung von Kohlenhydraten bei *Oniscus asellus* (Isopoda) und *Polydesmus angustus* (Diplopoda). *Pedobiologia*, **21** : 19-29.

Bignell (D. E.), 1989. – Relative assimilations of ^{14}C-labelled microbial tissues and ^{14}C-plant fibre ingested with leaf litter by the millipede *Glomeris marginata* under experimental conditions. *Soil Biol. Biochem.*, **21** : 819-827.

Bocok (K. L.), 1963. – The digestion and assimilation of food by *Glomeris*. *In:* Doeksen (J.), Van Der Drift (J.) Eds., *Soil organisms*. Amsterdam, 85-91.

Crawford (C. S.), Minion (G. P.) & Boyers (M. D.), 1983. – Intima morphology, bacterial morphotypes, and effects of annual molt on microflora in the hindgut of the desert millipede, *Orthoporus ornatus* (Girard) (Diplopoda: Spirostreptidae). *Int. J. Insect Morphol. and Embryol.*, **12** : 301-312.

Crossley (D. A.), 1977. – The roles of terrestrial saprophagous arthropods in forest soils: current status of concepts. *In*: Mattson (W. J.) Ed., *The role of arthropods in forest ecosystems*. Springer, Berlin, Heidelberg, New York, 49-56.

Deleporte (S.), 1988. – Étude expérimentale de l'ajustement entre le cycle de *Bradysia confinis* (Diptera: Sciaridae) et l'évolution du substrat trophique (litière de feuillus): importance des microorganismes. *Acta Oecol., Oecol. Gener.*, **9** : 13-35.

François (C.), Rafidison (Z.), Villemin (G.), Toutain (F.) & Andreux (F.), 1986. – The accumulation and fate of brown pigments in leaves from a litter of *Fagus silvatica* L.: a morphological and chemical study. *In: Proc. of 7th Symp. Environ. Biochem.*

Gere (G.), 1956. – The examination of the feeding biology and the humificative function of Diplopoda and Isopoda. *Acta Zool. Acad. Sci. Hung.*, **6** : 257-271.

Hanlon (R. D. G.), 1981 *a*. – Some factors influencing microbial growth on soil animal faeces. I. Bacterial and fungal growth on particulate oak leaf litter. *Pedobiologia*, **21** : 257-263.

HANLON (R. D. G.), 1982*b*. – Some factors influencing microbial growth on soil animal faeces. II. Bacterial and fungal frowth on soil animal faeces. *Pedobiologia*, **21** : 264-270.

HANLON (R. D. G.) & ANDERSON (J. M.), 1980. – Influence of macroarthropod feeding activities on microflora in decomposing oak leaves. *Soil Biol. Biochem.*, **12** : 255-261.

INESON (P.) & ANDERSON (J. M.), 1985. – Aerobically isolated bacteria associated with the gut and faeces of the litter feeding macroarthropods *Oniscus asellus* and *Glomeris marginata*. *Soil Biol. Biochem.*, **17** : 843-849.

JAMBU (P.), JUCHAULT (P.) & MOCQUARD (J. P.), 1988. – Étude experimentale de la contribution du crustacé isopode *Oniscus aselus* à la transformation des litières forestières sous chêne sessile. *Pedobiologia*, **32** : 147-156.

JONGERIUS (A.) Ed., 1964. – *Soil micromorphology*. Elsevier, Amsterdam, 540 p.

KILBERTUS (G.) & VANNIER (G.), 1978. – Étude des relations microfaune-microflore du sol en prenant comme exemple quatre espèces d'insectes Collemboles. *Comptes-rendus du 103e Congrès National des Sociétés Savantes Nancy*, 1978, Fascicule 3 : 63-75.

KILBERTUS (G.) & VANNIER (G.), 1979. – Microbial analysis and weight estimation of faeces produced by four sympatric Collembola species in forest litter. *Rev. Écol. Biol. Sol.*, **16** : 169-180.

KILBERTUS (G.) & VANNIER (G.), 1981. – Relations microflore-microfaune dans la grotte de Sainte-Catherine (Pyrénées ariégeoises). II. Le régime alimentaire de *Tomocerus minor* (Lubbock) et *Tomocerus problematicus* Cassagnau (Insectes, Collemboles). *Rev. Écol. Biol. Sol*, **18** : 319-338.

MARCUZZI (G.) & TURCHETTO LAFISCA (M.), 1976. – Contribute to the knowledge of the digestive enzymes of some litter feeding animals. Oligosacharases. *Rev. Écol. Biol. Sol*, **13** : 401-406.

MCBRAYER (J. F.), 1973. – Exploitation of deciduous leaf litter by *Apheloria montana* (Diplopoda: Eurydesmidae). *Pedobiologia*, **13** : 90-98.

NICHOLSON (P. B.), BOCOK (K. L.) & HEAL (O. W.), 1966. – Studies on the decomposition of the faecal pellets of a millipede (*Glomeris marginata* (Villers)). *J. Ecol.*, **54** : 755-766.

NIELSEN (C. O.), 1962. – Carbohydrases in soil and litter invertebrates. *Oikos*, **13** : 200-215.

NUNEZ (F. S.) & CRAWFORD (C. S.), 1976. – Digestive enzymes of the desert millipede *Orthoporus ornatus* (Girard) (Diplopoda: Spirostreptidae). *Comp. Biochem. Physiol.*, **55** A : 141-145.

REISINGER (O.), KILBERTUS (G.) & MANGENOT (F.), 1971. – Electron microscopical studies on the first stages of humification. *Humus and Planta*, V : 43-45.

SAUR (E.) & PONGE (J. P.), 1988. – Alimentary studies on the collembolan *Paratullbergia callipygos* using transmission electron microscopy. *Pedobiologia*, **31** : 355-379.

TAJOVSKÝ (K.), 1989. – *Millipedes (Diplopoda) and woodlice (Oniscidea) in the secondary successional row of the brown soils*. Thesis, České Budějovice, Czech., 172 p.

TAJOVSKÝ (K.), ŠANTRŮČKOVÁ (H.), HÁNĚL (L.), BALÍK (V.) & LUKEŠOVÁ (A.), in press. – Decomposition of faecal pellets of the millipede *Glomeris hexasticha* (Diplopoda) in forest soil. *Pedobiologia*.

TAYLOR (E. C.), 1982. – Fungal preference by a desert millipede *Orthoporus ornatus* (Spirostreptidae). *Pedobiologia*, **23** : 331-336.

TAYLOR (E. C.) & CRAWFORD (C. S.), 1982. – Microbial gut symbionts and desert detritivores. *Sci. Rev. Arid Zone Res.*, **1** : 37-52.

TOUTAIN (F.), 1981. – Les humus forestiers – Structures et modes de fonctionnement. *Rev. Forest. Fr.*, **6** : 449-477.

TOUTAIN (F.), 1987. – Activité biologique des sols, modalités et lithodépendance. *Biol. Fertil. Soils*, **3** : 31-38.

URBÁŠEK (F.) & TAJOVSKÝ (K.), in press. – The influence of food and temperature on enzymatic activities of the millipede *Glomeris hexasticha* (Diplopoda). *Rev. Ecol. Biol. Sol*, **28** (2).

VAN DER DRIFT (J.), 1975. – The significance of the millipede *Glomeris marginata* (Villers) for oak-litter decomposition and approach of its part in energy flow. *In*: VANĚK (J.) Ed. : *Progress in soil zoology*. Academia, Praha, 293-298.

VILLEMIN (G.) & TOUTAIN (F.), 1987. – Méthode de fixation d'échantillons organo-minéraux des sols pour la microscopie électronique à transmission. *In*: FEDOROFF (N.) *et al.* Eds.: *Micromorphologie des Sols – Soil Micromorphology*. Paris, 43-48.

WEBB (D. P.), 1977. – Regulation of deciduous forest litter decomposition by soil arthropod faeces. *In*: MATTSON (W. J.) Ed.: *The role of arthropods in forest ecosystems*. Springer, Berlin, Heidelberg, New York, 57-69.